Research Journal of Parasitology

ISSN 1816-4943

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Parasitology

ISSN 1816-4943
DOI: 10.3923/jp.2016.73.78

Research Article
Molecular Characterization of *Setaria equina* Infecting Donkeys (*Equus asinus*) from Egypt

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Abstract

Objective: Since few molecular data about *Setaria equina* are available, as this study proposed in order to investigate the molecular characteristics and the phylogenetic position of *S. equina* isolates from donkeys which was previously studied in horses. **Methodology:** The DNA was extracted from adult worms of *S. equina* found in the peritoneal cavity of donkeys slaughtered at Giza Zoo Abattoir, Egypt. A fragment length of the cytochrome oxidase subunit 1 (COX 1) gene (683 bp) was amplified using PCR. Purified PCR product was sequenced. Sequences were aligned with those published on GenBank and subsequently, the phylogenetic tree was constructed. **Results:** Results of the BLAST search showed that our isolates from donkeys are homologous (99% identity) with that from horses (AJ544873). Phylogenetic analysis exhibited the sister relationship between *S. equina* isolates from both donkeys and horses which illustrates the conspecificity between them and suggesting the cross transmission of this parasite species among different equids. **Conclusion:** The genetic relevance of this parasite to the other filarial worms was discussed in details. This is the first report about the molecular identification of *S. equina* infecting donkeys from Egypt.

Key words: *Setaria equina*, donkey, PCR, phylogenetic analysis

Received: May 13, 2016 Accepted: May 30, 2016 Published: September 15, 2016

Citation: Ibrahim Abbas, Moustafa Al-Araby and Yara Al-Kappany, 2016. Molecular characterization of *Setaria equina* infecting donkeys (*Equus asinus*) from Egypt. Res. J. Parasitol., 11: 73-78.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Setaria equina (family: Onchocercidae, subfamily: Setariinae) is a nematode filarial parasite commonly found in the peritoneal cavity of equines in different geographical regions of the world. The infection is believed to be transmitted by *Aedes* or *Culex* mosquitoes (Levine, 1985; Arundel, 1978; Coleman *et al*., 1985). The parasite is might erratically incriminated in the eye, brain, spinal medulla, vaginal sac and testicles of horses (Soulsby, 1982; Rodriguez-Vivas *et al*., 2000; Yadav *et al*., 2006; Gangwar *et al*., 2008; Kornas *et al*., 2010). Although infections are typically benign, pathologies of the eye and central nervous system (cerebrospinal nematodiasis) have been attributed to *S. equina* (Frauenfelder *et al*., 1980). Moreover, equine testicular lesions were noted (Marino *et al*., 2009). Taylor *et al*. (2001) recorded *S. equina* as one of the zoonotic species causing human disease.

The adult worms are thread-like ranged from 5-13 cm in length with the males being the smaller (Levine, 1985). The sheathed microfilaria worms are approximately 250-269 µm and found in the blood (Yeargan *et al*., 2009). Few studies are concerned with the prevalence of *S. equina* in horse populations. Low incidences were reported, 2% (Mfitilodze and Hutchinson, 1989), 4.4% (Al Anazi and Alyousif, 2011) and 8% (Gawor, 1995), while moderate prevalence rate (15%) was noted in Turkey (Oge *et al*., 2003). In Egypt, the infection with *Setaria equina* has been recorded (Abu El-Magd and Ahmed, 1994; Abdel-Wahab and Ashour, 1999; Marzok and Desouky, 2009). Still in Egypt, a high prevalence rate (40.08%) was reported in donkeys (Ahmed *et al*., 2011).

Nearly, 43 species of the genus *Setaria* have been identified so far in the world. The morphological characters alone are insufficient to establish the phylogenetic relationships and position of filarial nematodes (Chabaud and Bain, 1994), due to their similar morphological characteristics (Yatawara *et al*., 2007). Phylogenetic analysis is needed to evaluate and confirm the morphological description and taxonomy of *Setaria* species. It was reported that *S. digitata* and *S. labiatopapillosa* appeared to be sisters, as do *S. equina* and *S. tundra* (Yatawara *et al*., 2007). While, Alasaad *et al*. (2012) add *S. cervi* to the group of *S. digitata* and *S. labiatopapillosa* based on COX 1 sequence.

Setaria equina infecting horses (*Equus caballus*) from Italy was studied molecularly (Casiraghi *et al*., 2004). An important point of debate is that if *S. equina* infecting horses and those infecting donkeys are conspecific. As well as it is known that no molecular studies have been carried out to characterize this parasite species from donkeys and to determine its phylogenetic relationship with the other filarial worms.

This study was planned in order to find the molecular characteristics and the phylogenetic position of *S. equina* infecting donkeys, slaughtered at Giza Zoo Abattoir, Egypt, based on partial sequences of the mitochondrial cytochrome c oxidase subunit 1 (COX 1) gene. This study may help in future epidemiological prospects for developing control regimens against *S. equina*.

MATERIALS AND METHODS

Samples collection: Three adult nematode worms were collected during peritoneal cavities inspection of the slaughtered donkeys at Giza Zoo Abattoir, Egypt. These worms were identified as *S. equina* according to their morphological characteristics (Soulsby, 1982). Nematodes were washed 3 times by phosphate buffer saline and preserved in ethanol 70% until the DNA extraction.

DNA extraction: Genomic DNA was extracted from each worm using the standard phenol/chloroform technique (Sambrook *et al*., 1989).

PCR amplification: The PCR amplification of a fragment of the COX 1 gene was carried out in 35 µL final PCR mixture contained 2 µL of template DNA, 1 µL (25 µM) of each primer (Casiraghi *et al*., 2001) COX 1 intF (5'-TGATTGGTGGTTTTGGTAA-3') and COX 1 intR (5'-ATAAGTACGAGTATCAATATC-3'), 0.7 µL (10 mM) dNTP mix, 3.5 µL of taq buffer (10x), 0.35 µL Taq polymerase (5Prime Perfect Taq^{Tm}) and 26.45 µL nuclease free water. Negative control with no DNA was used. For amplification, samples were subjected to the following thermal profile, initial denaturation (94°C for 4 min) followed by 30 cycles of each of denaturation (94°C for 1 min), annealing (52°C for 1 min) and extension (72°C for 50 sec), then a final extension step (72°C for 5 min).

The resulted PCR products were subjected to gel electrophoresis using 1% agarose gel stained with ethidium bromide. Bands on gel nearly at 680 bp were purified with QIA quick PCR purification column (QIAGEN, GmbH, Hilden, Germany), then commercially sequenced using the PCR primers as sequencing primer. Searching for sequence similarity of samples was done using NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The obtained sequences were aligned with those published on the GenBank (Table 1) and the phylogenetic tree was constructed using the software Mega (version 6).

Table 1: Retrieved COX 1 sequences from GenBank which used for the phylogenetic tree construction

Parasite	Animal	Country	Accession No.	References
Onchocerca volvulus	Cattle	Cameron	KC167355	Eisenbarth *et al.* (2013)
Onchocerca ochengi	Cattle	Cameron	KC167351	Eisenbarth *et al.* (2013)
Onchocerca gutturosa	Cattle	Cameron	AJ271617	Casiraghi *et al.* (2001)
Onchocerca gibsoni	Cattle	Australia	AJ271616	Casiraghi *et al.* (2001)
Onchocerca skrjabini	Deer	Japan	AM749270	Ferri *et al.* (2009)
Onchocerca eberhardi	Deer	Japan	AM749268	Ferri *et al.* (2009)
Onchocerca takaokai	Pig	japan	AB972360	Uni *et al.* (2015)
Onchocerca dewittei japonica	Pig	Japan	AB518875	Fukuda *et al.* (2010)
Onchocerca suzukii	Japanese Serow	Japan	AM749277	Ferri *et al.* (2009)
Dirofilaria repens	Mosquitoes	Germany	KF692102	Kronefeld *et al.* (2014)
Setaria digitata	Cattle	Sri Lanka	EF174426	Yatawara *et al.* (2007)
Setaria digitata	Cattle	Sri Lanka	EF174423	Yatawara *et al.* (2007)
Setaria cervi	Red deer	Italy	JF800924	Alasaad *et al.* (2012)
Setaria labiatopapillosa	Cattle	Italy	AJ544872	Casiraghi *et al.* (2001)
Setaria equina	Horse	Italy	AJ544873	Casiraghi *et al.* (2001)
Setaria tundra	Unknown	Italy	AJ544874	Casiraghi *et al.* (2001)
Setaria tundra	Mosquitoes	Germany	KF692105	Kronefeld *et al.* (2014)
Setaria tundra	Mosquitoes	Germany	KF692103	Kronefeld *et al.* (2014)
Setaria tundra	Roe deer	France	AM749298	Ferri *et al.* (2009)
Loa loa	Human	Unknown	HQ186250	McNulty *et al.* (2012)
Wuchereria bancrofti	Human	Mali	JN367461	Ramesh *et al.* (2012)
Brugia malayi	Human	Unknown	AF538716	Ghedin *et al.* (2007)

RESULTS

For all the three examined *S. equina* samples from donkeys, PCR amplification of a fragment of the COX 1 gene resulted in gel bands at 680 bp length, while the negative controls gave no bands.

Sequence polymorphism: Sequences from the three investigated *S. equina* worms were identical. Results of the BLAST search showed a 99% identity between the revealed *S. equina* from donkeys in this study and those from horses in Italy which deposited in GenBank under the accession No. AJ544873 with 7 nucleotide substitution C50T, A61T, T104C, C202G, A289G, A564T and A577G (Fig. 1). A deletion was noted at the site 592 of donkeys' *S. equina* sequence. Concerning the relationship with the other *Setaria* species, there were nearly similar identity percents between their sequences and those reported in this study. A 90% identity was noted with *S. tundra* (AM749298), *S. labitopapillosa* (AJ544872) and *S. cervi* (JF800924), while 89% identity was found with *S. digitata* (EF174426).

Phylogenetic analysis: As shown in Fig. 2, data emerged from the phylogenetic tree based on COX 1 sequences showed that the genus *Setaria* shared the same calde with *Onchocerca* and *Dirofilaria*, while the *Wuchereria*, *Brugia* and *Loa loa* formed separate clades. Moreover, *Setaria* species are monophyletic and localized in 2 sister groups, the first including *S. equina* and *S. tundra* while, the other group consisted of *S. digitata*, *S. cervi* and *S. labitopapillosa*. The revealed *S. equina* from donkeys in the present study was found in the same sister group with *S. equina* recovered from horses.

DISCUSSION

Filarioid nematodes affect millions of people and animals allover the world bringing up to major health hazards and economic losses (World Health Organization, 2006).

Studying the molecular characteristics of filarial worms is important to emphasis their identification and taxonomy since their similar morphological characteristics weakened the evolutionary pattern (Yatawara *et al.*, 2007). Another considerable point for studying the genetic relationship between the filarioid worms is the cross antigenicity between them. Antigens from *Setaria* species have the potential for immunodiagnosis of human filariosis. Cross reaction was found between *S. equina* antigens and antibodies in the sera of *Wuchereria bancrofti* infected patients, especially in the chronic infected subjects (Bahgat *et al.*, 2011).

Like what have been previously stated by Casiraghi *et al.* (2004) and Yatawara *et al.* (2007), these results showed that the filarial worms formed 2 main clades according to their phylogenetic position, the genus *Setaria* is a member of a large clade along with *Onchocerca* and *Dirofilaria*, while, *Wuchereri bancrofti*, *Brugia malayi* and *Loa loa* are located in a separate clades. Moreover, both *Wuschereria bancrofti* and *Brugia malayi* are appeared to be sisters. In this study,

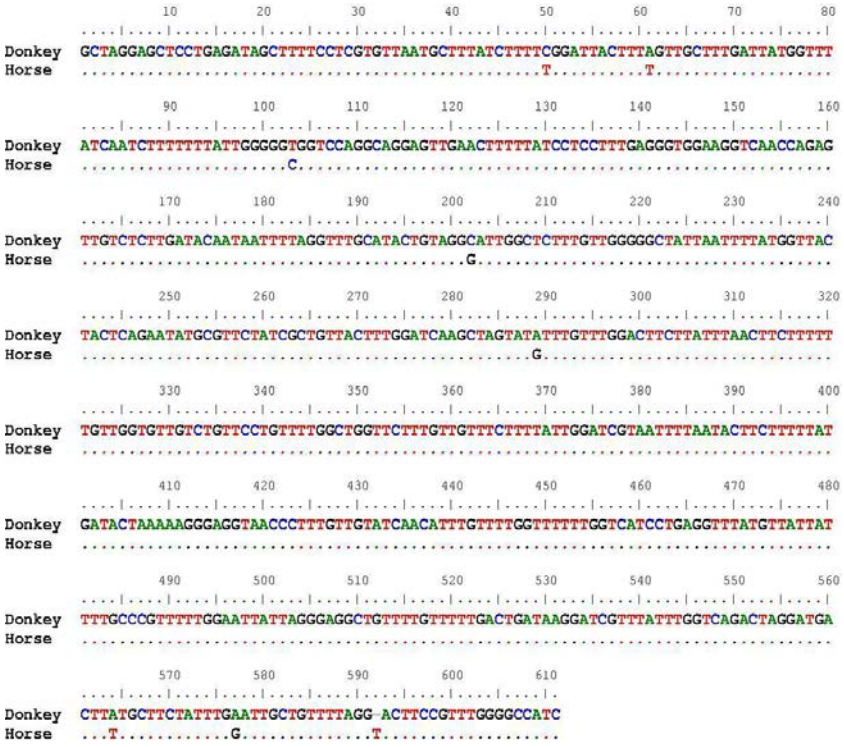

Fig. 1: Alignment of the partial COX 1 nucleotide sequence of *Setaria equina* isolated from donkey with that from horse (AJ544873). Seven nucleotide substitutions are noted: C50T, A61T, T104C, C202G, A289G, A564T and A577G. There is a deletion at the site 592 in the donkey`s sequence

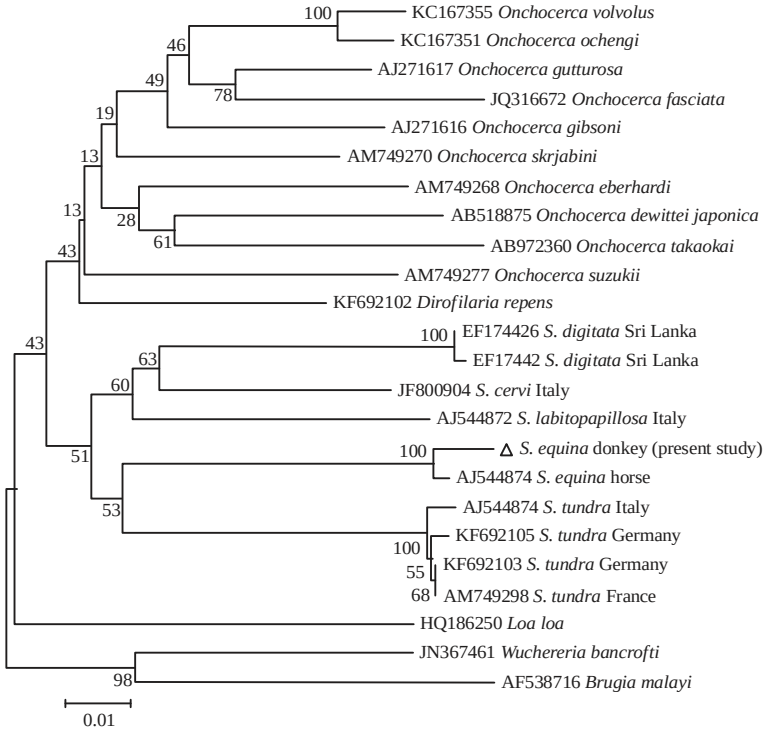

Fig. 2: Phylogenetic tree inferred from partial COX 1 nucleotide sequences using the neighbor joining method in the software Mega 6 and showing a sister relationship between isolates of *Setaria equina* from both donkeys and horses. Scale bar indicates the proportion of sites changing along each branch

Dirofilaria does not share the same branch with *Setaria*, opposite to what have been stated by Jayasinghe and Wijesundera (2003).

Concerning the genus *Setaria*, the inferred data from this study confirmed the results of the phylogenetic position within *Setaria* species which previously recorded (Casiraghi *et al*., 2004; Yatawara *et al*., 2007; Alasaad *et al*., 2012). All *Setaria* species are found in the same phyletic group in which *S. equina* and *S. tundra* appeared to be sisters, as do *S. digitata*, *S. cervi* and *S. labitopapillosa*.

Setaria equina is reported globally. The molecular characteristics and phylogenetic position of *S. equina* was firstly described by Casiraghi *et al*. (2004). They collected the samples from horses in Italy. This study is the first report about the molecular characterization of *S. equina* infecting donkeys. Results showed that both of the revealed *S. equina* isolates either from horses or donkeys are sisters and this confirmed the conspeicificty between them and in turn, this species could be crossly transmitted between horses and donkeys.

CONCLUSION

In conclusion, a further study with frequent number of *S. equina* specimens from different equides (horses, donkeys and mules) as well as the arthropode vectors should be carried out in order to enhance the understanding about the transmission of this parasite and its relation with the other members of the genus *Setaria* and with the other filarial worms.

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